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Open Sesame! Coxsackieviruses Conspire to Trespass the Tight Junctional Gate

In the January 13 issue of *Cell*, Coyne and Bergelson describe an "Open sesame!" strategy developed by coxsackieviruses to invade the organism through the intestinal epithelium. The strategy involves coopting intrinsic signaling abilities of the apical GPI-anchored protein DAF to open the tight junction barrier, gain access to the primary receptor CAR, and activate virus internalization by a caveolin-dependent pathway.

Pathogens intent on invading an organism must overcome imposing epithelial barriers, i.e., thick cornified skin on the outside and the tightly sealed single epithelial layers of digestive and respiratory mucosae on the inside. In a recent issue of *Cell*, Coyne and Bergelson (2006) present a wonderfully detailed picture of a cunning strategy developed by a subgroup of coxsackieviruses B (CVB) to penetrate intestinal epithelia. Like in "Ali Baba and the Forty Thieves," the viruses utilize a magic "sesame" key to open the tight junctional gate and gain access to the cellular treasures that allow their replication. This magic key is decay-accelerating factor (DAF), an apical GPI-anchored protein that, presumably because of its critical function regulating the deposition and activation of complement on the cell surface, its high relative mobility in the plane of the membrane, and obligate expression on the apical surface of polarized epithelial cells, has repeatedly been a target for picornavirus adhesion. Bergelson's group had previously shown that coxsackieviruses display Darwinian skills in how they develop variants able to bind DAF after growth in cells that express DAF (Milstone et al., 2005).

The role of DAF in virus entry is far from passive (Figure 1). DAF is targeted to its normal location at the apical surface of intestinal cells by its GPI-anchor and the lipid rafts associated with it (Rodriguez-Boulan et al., 2005). Although DAF is a GPI-anchored protein, it has been shown to interact with a variety of proteins on nucleated cells including tyrosine kinases. The manner in which DAF interacts with these kinases is not clear (Nicholson-Weller and Wang, 1994). The authors convincingly show that DAF and raft clustering by CVB or by antibodies specifically activates two cellular signals: the nonreceptor tyrosine kinase Abl and Src family kinases, including Fyn. Activated Abl is known to mediate actin remodeling, apparently through Rac activation in the case of DAF stimulation by CVB (as shown by the authors). The purpose of actin remodeling is to generate a mechanism to transport the virus to its primary receptor, CAR (coxsackie-adenovirus receptor), an integral transmembrane protein of the immunoglobulin family, which is a structural component of TJ (Cohen et al., 2001). Arrival of CVB promotes junctional disassembly, reflected in decreased transmonolayer electrical resistance, apparently by mechanical interference of the virus with CAR homophilic adhesion rather than through effects downstream of DAF activation, such as disruption of the actin cytoskeleton—a strategy used by enteropathogenic *E. coli* (Vogelmann et al., 2004). The sophisticated strategy used by CVB viruses to open TJ resembles the use of adenovirus fibers by adenoviruses to dissociate CAR (Walters et al., 2002), although in the latter case the viruses may depend on junction opening by epithelial injury or inflammation for the initial access to CAR.

Coyne and Bergelson show that the binding of CVB particles to CAR at the TJ is required for capsid destabilization (Figure 1, shown as a change in the color of CVB particles at the TJ), a step important in the reproduction of adenoviruses, reoviruses, and picornaviruses, as it exposes binding sites in the viruses for secondary receptors and it allows the release of the viral genetic material from endosomes. Secondary receptors have been identified for some viral families—for example, the $\alpha v\beta 3$/ $\alpha v\beta 5$ integrins used by some adenoviruses—but much remains unknown about the capsid destabilization step. Coyne and Bergelson confirm that DAF activation does not promote capsid destabilization (Milstone et al., 2005), which continues to be a function of the interaction with the primary receptor CAR, and additionally show that capsid destabilization, which takes place prior to internalization, is not sufficient for virus endocytosis. The study does add DAF to the group of coreceptors, which also includes CD46 for other coxsackievirus subfamilies.

Interaction of CVB with CAR leads somehow to internalization apparently through a caveolin-1 pathway, as demonstrated by interference with a dominant-negative caveolin mutant. The activation of caveolin-1 for internalization appears to be a downstream effect of DAF clustering. The Src family kinase Fyn is phosphorylated on Tyr^{418} 10 min after virus binding to DAF, and specific inactivation of Fyn with pharmacologic and RNAi approaches inhibited CVB entry. Fyn phosphorylates caveolin-1 at Tyr^{14}; P-caveolin can then be detected by immunofluorescence in vesicles containing CVB at the level of the TJ. Caveolae have been recently shown to play a key role in virus- and toxin-induced internalization of SV40 and cholera toxin in nonpolarized cells (Pelkmans and Helenius, 2003), but little is known about the role of caveolae in virus internalization in polarized cells. Although caveolin-1 may be found at the apical surface of epithelial cells, typical morphological caveolae are only basolateral in most epithelial cells; to our knowledge, caveolae have not yet been described at the level of tight junctions (Rodriguez-Boulan et al., 2005). The nature of the endocytic vesicles that internalize CVB and caveolin was addressed but not fully solved by the authors. Inhibitors of clathrin-mediated endocytosis and dynamin did not prevent viral internalization. As dynamin is involved in the internalization of both clathrin-coated vesicles and caveolae, the CVB internalization route may be a novel one, perhaps related to the clathrin-independent carriers (CLIC), which use utilize a clathrin- and dynamin-independent mechanism

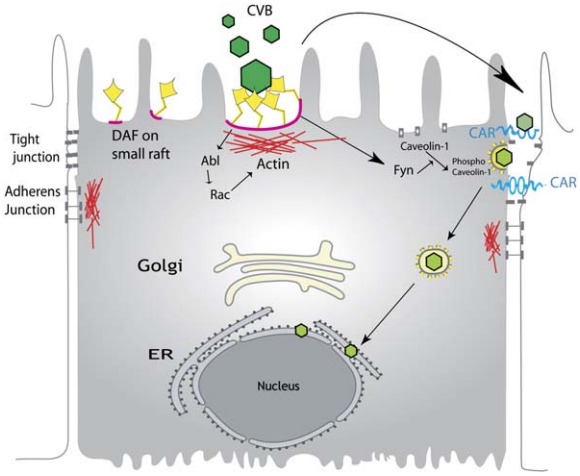

Figure 1. Coxackievirus B with Affinity for Decay-Accelerating Factor Clusters Several DAF Molecules at the Apical Membrane of the Intestinal Cell, Thus Converting the Associated Small Lipid Rafts into Larger Functional Rafts

This clustering process activates downstream tyrosine kinase Abl, which remodels actin via Rac, necessary for driving decay-accelerating factor (DAF) bound coxackievirus B (CVB) to the tight junctions. Here, the viruses are transferred from DAF to their primary receptor, CAR; this dissociates CAR's homotypic interactions with CAR in the neighboring cell. Binding to CAR leads to changes in capsid conformation (dark to light green). DAF clustering also activates the Src tyrosine kinase Fyn, which phosphorylates and activates caveolin-1, which promotes virus internalization into vesicles (caveolae?) that exclude CAR and DAF. An hour and a half post infection, viral particles localize to a perinuclear region rich in ER.

sensitive to cholesterol depletion (Kirkham and Parton, 2005). However, this mechanism was described for GPI-anchored proteins and involves cdc42; CVB vesicles exclude DAF and do not seem to require cdc42. Electron microscopy studies will be helpful to clarify the morphological features of the endocytic vesicle that internalizes CVBs. Very little is known about later stages of the internalization route of CVB, which appears to include an ER step, although recent evidence suggests that caveolin-1 might be a determinant of this route (Pelkmans and Helenius, 2003).

This exciting and very well documented paper highlights the extremely dynamic properties of intercellular junctions, believed just a decade ago to be simple passive restraining devices. The CVB mechanism is reminiscent of strategies that leukocytes use to cross endothelial cell adherens junctions at sites of inflammation. Leukocyte $\alpha 4\beta 1$ integrins cluster VCAM-1 molecules on the apical endothelial surface, leading to Rac-1 activation and loosening of the junctions, possibly via phosphorylation of vascular endothelial cell-specific cadherin (VE-cadherin), a process that should facilitate leukocyte transmigration (Wittchen et al., 2005). At the endothelial junction, homophilic engagement of leukocyte and endothelial PECAM disrupts homophilic PECAM-PECAM interactions between endothelial cells (analogous to CAR-CAR interactions between epithelial cells) and triggers the recruitment of membrane vesicles from an intracellular perijunctional depot that surround the leukocyte with PECAM-bearing membrane and guide it across the junction (Mamdouh et al., 2003), reminiscent of internalization of CVB into a membrane bound compartment at the junction. The study by Coyne and Bergelson provides a beautiful example of the well-demonstrated usefulness of viruses to elucidate important cell biological processes. It has important implications for epithelial polarity studies, as the route followed by CVB through the tight junction might also be used by cellular proteins attempting to cross the tight junction barrier during transcytosis.

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